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Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract

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Abstract

The ethanol extracts from 24 samples plant species commonly found in Thailand were investigated and compared on their antioxidant activity by ABTS assay. The ethanol extract from the leaves of guava (*Psidium guajava*) showed the highest antioxidant capacity with the TEAC value of 4.908 ± 0.050 mM/mg, followed by the fruit peels of rambutan (*Nephelium lappaceum*) and mangosteen (*Garcinia mangostana*) with the TEAC values of 3.074 ± 0.003 and 3.001 ± 0.016 mM/mg, respectively. The further investigation of guava leaf extracts from different solvents; *n*-hexane, ethyl acetate, *n*-butanol, and methanol, was examined using ABTS and FRAP assays. The total phenolic content was done by Folin–Ciocalteu reaction. The results indicated that the methanol fraction possessed the highest antioxidant activity, followed by the butanol and ethyl acetate fractions, respectively. The hexane fraction showed the lowest antioxidant activity. The results demonstrated that the mechanism of antioxidant action of guava leaf extracts was free radical scavenging and reducing of oxidized intermediates. The phenolic content in guava leaf fraction played a significant role on the antioxidant activity via reducing mechanisms.

Keywords: Antioxidant activity; Extract; ABTS; FRAP; Phenolic content; Guava

1. Introduction

It is well known that reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. Tissue damage resulting from an imbalance between ROS-generating and scavenging systems has been implicated in the pathogenesis of a variety of disorders, including degenerative disorders of the CNS, such as Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension, AIDS and aging (Halliwell & Gutteridge, 1998; Mantle, Eddeb, & Pickering, 2000).

Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen. They act by one or more of the following mechanisms: reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. Epidemiological studies have shown that many phytonutrients of fruits and vegetables might protect the human body against damage by ROS. The consumption of natural antioxidant phytochemicals was reported to have potential health benefits (Di Carlo, Mascolo, Izzo, & Capasso, 1999; Pulido, Bravo, & Saura-Calixto, 2000; Sumino, Sekine, Ruangrungsi, Igarashi, & Ikegami, 2002). In recent years, there has been a considerable interest in finding natural antioxidants from plant materials. The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease (Kinsella, Frankel, German, & Kanner, 1993; Terao & Piskula, 1997), and to retard lipid oxidative rancidity (Duthie, 1993). In addition, the use of synthetic antioxidants has

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been questioned because of their toxicity (Valentao et al., 2002). Therefore, there have been numerous researches on these bioresources to seek for potential natural and possibly economic and effective antioxidants to replace the synthetic ones.

Thailand, as a tropical country, shows on amazing diversity of plants species. Some of them have long been used as traditional medicines. Many of them were reported to have various desirable activities (Okonogi & Murakoshi, 1994; Okonogi, Murakoshi, & Sekine, 1993; Sekine et al., 1993). In the present study, we collected 24 plant species that were commonly found in the northern region of Thailand. The ethanol crude extracts from the interesting parts, such as the leaf, the stem, the fruit pulp and the fruit peel, were evaluated and compared for their antioxidant activities. The plant whose ethanol crude extract showed the highest antioxidant potential was further evaluated for its mechanism of antioxidant activity and phenolic content.

2. Materials and methods

2.1. Chemicals

Gallic acid, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), butylated hydroxytoluene (BHT), quercetin (QCT), ferric chloride ($FeCl_3 \cdot 6H_2O$), and potassium persulfate were from Sigma (MO, USA). β-Carotene and 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ) were from Fluka Chemicals (Buchs, Switzerland). Trolox was from Aldrich Chemical Company (Steinheim, Germany). Ethanol, methanol, *n*-hexane, ethyl acetate, *n*-butanol, and hydrochloric acid were from Merck (Darmstadt, Germany). All other reagents were of the highest quality grade available.

2.2. Plant material

Twenty four plant species were collected during April and September from the northern region of Thailand. Some of them are not indigenous to the area but grow wildly in Thailand. The collection sources of plant samples were different. Some were harvested from the plant gardens, the others were purchased from local markets, as shown in Table 1. Voucher specimens of certain plants were deposited in the Herbarium of the Faculty of Pharmacy, Chiang Mai University. The interesting parts of plant samples were carefully separated and cut into small thin pieces and dried at 50 °C for 2 days. The dried plant samples were ground into fine powder and stored in a vacuum desiccator at 4 °C for further study.

2.3. Preparation of extracts

2.3.1. Ethanol crude extracts

The dried powder of each plant sample was extracted by maceration with ethanol (100 g dried power sample/500 ml of 95% ethanol) for 48 h (×3) at room temperature. The filtrates of each time were pooled and the solvent was removed under vacuum at 45 °C using a rotary evaporator. The obtained crude extracts were stored in a desiccator at

Table 1
The details of plants used in this study

Scientific name	Local name	English name	Family	Main traditional uses
Andrographis paniculata[a]	Fa-talai-chon	–	Acanthaceae	Antipyretic
Clausena lansium[a]	Ma-fai-jin	–	Rutaceae	–
Citrus hystrix[a]	Ma-krut	Lime	Rutaceae	Carminative
Cocos nucifera[a]	Ma-prao	Coconut	Palmae	Antimicrobial
Cymbopogon citratus[a]	Ta-krai	Lemon grass	Gramineae	Antibacterial
Dregea volubilis[a]	Puck-huan-muu	–	Asclepladaceae	–
Garcinia mangostana[a]	Mung-kut	Mangosteen	Guttiferae	Antidiarrhea
Gymnema inodorum[a]	Chiang-da	–	Asclepladaceae	Antihyperglycemia
Hylocereus undatus[b]	Kaew-mang-korn	Dragon Fruit	Cactaceae	–
Hyptis suaveolens[a]	Mang-luk-ka	Wild spikenard	Labiatae	Antibacterial
Lansium domesticum[b]	Long-kong	Lansium	Meliaceae	–
Leucaena leucocephala[a]	Ka-tin	Leucaena	Leguminosae	Relieves diarrhea
Marsdenia glabra[b]	Puck-saew	–	Asclepladaceae	Antipyretic
Mentha cordifolia[a]	Sa-ra-nae	Kitchen mint	Labiatae	Carminative
Musa sapientum[b]	Kloay	Banana	Musaceae	Antiulcer
Nephelium lappaceum[b]	Ngo	Rambutan	Sapindaceae	–
Ocimum basilicum[a]	Ho-ra-pa	Basil	Labiatae	Carminative
Ocimum gratissimum[b]	Yee-ra	Tree basil	Labiatae	Carminative
Ocimum sanctum[b]	Ka-prao	–	Labiatae	Carminative
Oroxylum indicum[a]	Pae-ka	Damocles tree	Bignoniaceae	–
Passiflora foetida[b]	Sao-wa-rood	Passion fruit	Plasifloraceae	–
Piper sarmentosum[a]	Cha-plu	–	Piperaceae	Carminative
Psidium guajava[a]	Fa-rang	Guava	Myrtaceae	Antidiarrhea
Thunbergia laurifolia[a]	Rang-cherd	–	Thunbergiaceae	Antipyretic

[a] Plant samples were harvested from medicinal gardens.
[b] Plant samples were harvested from local markets.

4 °C. These ethanol crude extracts were subjected to ABTS assay for comparison of their antioxidant activities.

2.3.2. Fractionation of extracts

The dried powder plant sample whose ethanol crude extract showed the highest potential antioxidant activity after the comparison test by ABTS assay was selected and macerated in hexane for 48 h (×3) at room temperature. The residue after the third filtration was dried at room temperature for 24 h to ensure hexane was completely removed. The dried residue was further macerated in another three solvents as follows; ethyl acetate, butanol and methanol, respectively, in the same manner as hexane. The filtrates of the same solvent were pooled together. The solvent was removed under vacuum at 45 °C using a rotary evaporator. The extracts obtained from each solvent were kept in a desiccator at 4 °C for further study.

2.4. ABTS assay

This was done by using the ABTS free radical decolorization assay developed by Re et al. (1999) with some modification. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 750 nm. The plant extracts were separately dissolved in ethanol to yield a concentration of 1 mg/ml. An aliquot of 20 μl of ethanolic test solution of each sample was added to 180 μl of ABTS free radical cation solution. The absorbance, monitored for 5 min, was measured spectrophotometrically at 750 nm using a microtitre plate reader. All measurements were performed in triplicate. The free radical-scavenging activity of each sample was expressed as trolox equivalent antioxidant capacity (TEAC), which was obtained by comparing the absorbance change at 750 nm in a reaction mixture containing a sample of plant extract or test material with that containing trolox. This index is defined as the millimolar concentration of a trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). BHT and QCT were used as positive controls.

2.5. Determination of the reducing power

The reducing power was determined by using a ferric reducing ability of plasma (FRAP) assay described by Benzie and Strain (1996) with some modification. Briefly, the FRAP reagent contained 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6, was freshly prepared. The extracts were dissolved in ethanol at a concentration of 1 mg/ml. An aliquot of 20 μl test of solution was mixed with 180 μl of FRAP reagent. The absorption of the reaction mixture was measured at 595 nm by a microtitre plate reader. Ethanolic solutions of known Fe(II) concentration, in the range of 50–500 μM ($FeSO_4$), were used as calibration curve. The reducing power was expressed as equivalent concentration (EC). This parameter was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$. BHT and QCT were used as positive controls.

2.6. Determination of total phenolic content

Total phenolic content was analyzed, using the Folin–Ciocalteu method described by Sato et al. (1996) with some modification. Briefly, the plant extract was dispersed in ethanol to yield 1 mg/ml of test solution. An aliquot of 1 ml of test solution was diluted with 9 ml of distilled water. Afterwards, 200 μl Folin–Ciocalteu reagent and 600 μl of 2% sodium carbonate were added. The mixture was allowed to stand for 2 h at room temperature before the absorbance was measured spectrophotometrically at 750 nm. Gallic acid was used as the standard for the calibration curve. Total phenolic content of the sample was expressed as gallic acid equivalent concentration (mg/ml).

3. Results and discussion

3.1. General

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The amount of such compounds deposited in each part of the plant is usually different. In this study, 34 samples from 24 plant species, as shown in Table 1, were investigated for their antioxidant activity. The crude ethanol extracts of these samples were used for comparison of their antioxidant powers. The percentage yields of these ethanol crude extracts are presented in Table 2. They ranged from 1.12% to 17.52%. The fruit peel of *Citrus hystrix* gave the highest percentage yield whereas the stem of guava gave the lowest.

Several methods have been used for evaluation of the antioxidant activity of plants. Those are DPPH·-scavenging assay (Gamez et al., 1998), ABTS decolorization (Re et al., 1999), FRAP method (Benzie & Strain, 1996; Pulido et al., 2000), and β-carotene bleaching model (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998; Hidalgo, Fernandez, Quilhot, & Lissi, 1994; Jayaprakasha, Sigh, & Sakariah, 2001). Free radicals are a major cause of the propagation stage of the oxidation process. The high potential for scavenging of free radicals could inhibit spreading of oxidation. Hence, the comparative study, to seek for the highest potential antioxidant from the ethanol crude extracts was carried out by a free radical-scavenging method, using ABTS as free radical. This is an excellent method for determining the antioxidant activity of a broad diversity of substances, such as hydrogen-donating antioxidants or scavengers of aqueous phase radicals and of chain-breaking antioxidants or scavengers of lipid peroxyl

Table 2
The percentage yields and TEAC values of ethanol extracts

Plants	Part used	Yield (%) (w/w)	TEAC (mM/mg)[a]
Andrographis paniculata	Leaf	6.48	0.397 ± 0.022
	Stem	1.54	0.648 ± 0.047
Clausena lansium	Leaf	8.66	0.491 ± 0.011
Citrus hystrix	Leaf	7.42	0.781 ± 0.013
	Fruit peel	17.52	1.20 ± 0.034
Cocos nucifera	Fruit peel	5.80	1.53 ± 0.044
Cymbopogon citratus	Leaf	10.05	0.631 ± 0.057
	Stem	16.37	0.324 ± 0.014
Dregea volubilis	Leaf and stem	12.38	1.06 ± 0.087
Garcinia mangostana	Fruit peel	7.21	3.00 ± 0.016
Gymnema inodorum	Leaf	11.92	0.902 ± 0.052
	Stem	3.91	0.718 ± 0.027
Hylocereus undatus	Fruit peel	1.40	0.685 ± 0.021
Hyptis suaveolens	Leaf and stem	4.34	0.850 ± 0.022
Lansium domesticum	Fruit peel	5.71	0.507 ± 0.002
Leucaena leucocephala	Leaf	6.09	1.43 ± 0.007
	Pod	1.20	1.71 ± 0.033
Marsdenia glabra	Leaf	11.56	0.673 ± 0.044
Mentha cordifolia	Leaf	11.13	1.84 ± 0.030
	Stem	7.04	0.364 ± 0.006
Musa sapientum	Fruit peel	7.66	1.80 ± 0.038
Nephelium lappaceum	Fruit peel	10.68	3.07 ± 0.003
Ocimum basilicum	Leaf	9.82	0.877 ± 0.010
	Stem	4.85	0.783 ± 0.022
Ocimum gratissimum	Leaf	6.17	1.35 ± 0.014
Ocimum sanctum	Leaf	6.04	1.48 ± 0.030
	Stem	3.65	0.877 ± 0.004
Oroxylum indicum	Pod	7.50	0.506 ± 0.009
Passiflora foetida	Fruit peel	2.60	0.591 ± 0.023
Piper sarmentosum	Leaf	3.06	1.46 ± 0.020
Psidium guajava	Leaf	8.70	4.91 ± 0.050
	Stem	1.12	1.96 ± 0.016
	Fruit pulp	4.65	0.898 ± 0.008
Thunbergia laurifolia	Leaf	4.47	1.66 ± 0.011

[a] Values represent means ± SD ($n = 3$).

radicals (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995; Rice-Evans, Miller, & Paganga, 1996; Robert et al., 1999).

3.2. Antioxidant activity of the ethanol crude extracts

The results of primary screening of antioxidant activity of all ethanol crude extracts were expressed as TEAC value, as shown in Table 2. This value represented the mM trolox equivalents/mg extract. The antioxidant activities of the samples ranged widely from 0.324 to 4.91 mM trolox equivalents/mg extract. The leaves of guava (*Psidium guajava*) showed the highest antioxidant activity with a TEAC value of 4.91 ± 0.050 mM trolox equivalents/mg extract, followed by the fruit peels of rambutan (*Nephelium lappaceum*) and mangosteen (*Garcinia mangostana*) with TEAC values of 3.07 ± 0.003 and 3.00 ± 0.016 mM trolox equivalents/mg extract, respectively. The stem of *Cymbopogon citratus* showed the lowest antioxidant activity among the plant samples included in this study with the TEAC value of 0.324 ± 0.014 mM trolox equivalents/mg extract. When the leaf and the stem of each plant sample were compared, it was found that all plant extracts from the leaf exhibited higher antioxidant activity than did those from the stem. The extract from fruit peels also had a wide ranging antioxidant activity of 0.507–3.07 mM trolox equivalents/mg extract, depending on plant species. Among the fruit peel samples, the pericarp of rambutan showed the highest antioxidant capacity whereas that of *Lansium domesticum* showed the lowest. Of the 34 samples we analyzed, three showed extremely high antioxidant activity (TEAC values were above 3.0), 12 showed high antioxidant activity (TEAC values were below 3.0 but above 1.0), 15 showed moderate antioxidant activity (TEAC values were below 1.0 but above 0.5), and four showed low antioxidant activity (TEAC values were below 0.5). Within the extremely high antioxidant activity group, the leaves of guava exhibited the highest potential. According to the results from the ABTS assay, we could expect that one of the antioxidant mechanisms of guava leaf extract was via free radical-scavenging action. This result corresponded with the data reported by Qian and Nihorimbere (2004), even if different antioxidative test systems were used. In our study, three parts of the guava plant were examined for their antioxidant activities. The results showed that the antioxidant activities of each part were obviously different. Guava leaves showed the highest activity with the TEAC value of 4.91 ± 0.050 mM trolox equivalents/mg extract, whereas the stem and the fruit pulp were much lower with TEAC values of 1.96 ± 0.016 and 0.898 ± 0.008 mM trolox equivalents/mg extract, respectively. Therefore, the dried powder of guava leaves was selected for further investigation.

3.3. Antioxidant activity of guava leaf extracts

3.3.1. General

Qian and Nihorimbere (2004) reported that the extracts of guava leaves with 50% aqueous ethanol (1:10) ratio showed much higher antioxidant activities than did those with water. This suggested that the polarity of the active components in guava leaves was lower than water. As the polarity of methanol was slightly lower than water, this was considered to be one of the most suitable solvents for this extraction. On the other hand, the antioxidant activities of the guava leaf extracts from other lower polarity solvents, such as *n*-hexane, ethyl acetate and *n*-butanol, has not yet been reported elsewhere. Therefore, in this experiment the antioxidant activity of guava leaves was further studied by using several kinds of solvents for maceration, of different polarities: *n*-hexane, ethyl acetate, *n*-butanol and methanol, respectively. The extracts from each solvent were subjected to the ABTS test and FRAP assay. These two methods represented different mechanisms of antioxidant action. A sample possessing ABTS free radical-scavenging activity indicated that its mechanism of action was as a hydrogen donor and terminated the oxidation process by converting free radicals to more stable products, whereas a compound exhibiting a positive result in the FRAP assay was an electron donor and it terminated

the oxidation chain reaction by reducing the oxidized intermediates into the stable form.

3.3.2. ABTS scavenging activity

The TEAC values of guava leaves extracted from different solvents are shown in Fig. 1. All extracts possessed free radical-scavenging activity but at different levels. The highest activity was obtained from the methanol extract, with the TEAC value of 3.79 ± 0.003 mM trolox equivalents/mg extract, followed by the butanol extract and ethyl acetate extract with the TEAC values of 2.90 ± 0.023 and 2.65 ± 0.065 mM trolox equivalents/mg extract, respectively. The activities of the latter two fractions were above 70% of the methanol extract. The hexane extract exhibited the lowest scavenging action with the TEAC value of 1.06 ± 0.017 mM trolox equivalents/mg extract which was below 30% of the methanol extract. The antioxidant activity of the methanol extract was higher than that of the two positive controls, BHT and QCT, whereas the other two high activity fractions were a little lower. It was considered that the methanol fraction of guava leaves was a good source of potent natural antioxidant activity. Its high TEAC value, indicated that the mechanism of antioxidant action of this fraction was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to the stable forms.

3.3.3. FRAP reducing power

The principle of the FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so that they can act as primary and secondary antioxidants (Yen & Chen, 1995). Fig. 2 shows the reducing power of guava leaf extracts from different solvents. All extracts had reducing power but not at the same level. The result clearly indicated that the methanol extract of guava leaves had the highest reducing power with the EC value of 3.65 ± 0.038 mM/mg extract. This was much higher than that of BHT but a little lower than QCT. The ethyl acetate and butanol extracts showed lower activity with EC values of 1.15 ± 0.132 and 1.36 ± 0.032 mM/mg extract, respectively, which were about 31–37% that of the methanol extract. The lowest reducing property was obtained from the hexane fraction. From this point of view, it was confirmed that the methanol fraction of guava leaves possessed the potent antioxidant compounds. According to its high EC value, it could be considered that compounds in the methanol fraction were good electron donors and could terminate oxidation chain reactions by reducing the oxidized intermediates into the stable form.

3.4. Correlation of TEAC and EC values of guava leaf extracts

Fig. 3 shows a relationship between free radical-scavenging activity, as TEAC value, and the reducing power, as EC value, of guava leaf fractions extracted by four solvents with different polarities. It was clearly seen that the higher the polarity of solvent used in the extraction process, the higher was the antioxidant activity obtained. This led to the consideration that the most antioxidant active compounds in guava leaves should be of high polarity. The correlation between the two mechanism parameters (TEAC and EC values) was quite poor ($R^2 = 0.68$). This suggested that antioxidant components, existing in each fraction, possessed different predominant mechanisms of action. Moreover, the non-linear relationship between the two mechanism parameters might be due to other effects involving the nature of the active compounds existing in the guava leaves and their synergistic effects. Several antioxidants from plants have been reported to be polyphenolic compounds. Further study on phenolic content in each guava leaf fraction would be beneficial, leading to more

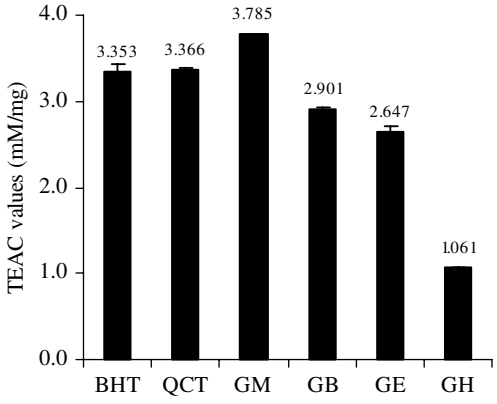

Fig. 1. Free radical-scavenging activity of guava leaf extracts from methanol (GM), butanol (GB), ethyl acetate (GE), and hexane (GH) in comparison with those of butylated hydroxyl toluene (BHT) and quercetin (QCT).

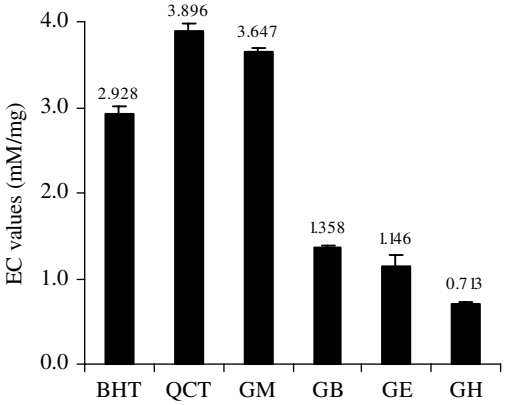

Fig. 2. Reducing power of guava leaf extracts from methanol (GM), butanol (GB), ethyl acetate (GE) and hexane (GH) in comparison with those of butylated hydroxyl toluene (BHT) and quercetin (QCT).

understanding of the active compounds related to their mechanism of action.

3.5. Total phenolic content

The total phenolic content of guava leaf fractions was reported as gallic acid equivalent concentration (mg/ml). The results showed that guava leaf fractions contained a mixture of phenolic compounds at different levels according to the polarity of solvent used in the extraction process, in the following order: methanol > butanol > ethyl acetate > hexane, as shown in Fig. 4. Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical-scavenging, lipid peroxidation and chelating of metal ions (Shahidi, 1997, chap. 11). Figs. 5 and 6 show the correlation between the phenolic content of the guava leaf fractions from different solvents, equivalent to gallic acid concentration vs. free radical-scavenging activity as TEAC values and reducing power as EC values, respectively. It was obvious that the phenolic content in the extracts showed a much higher correlation with reducing power ($R^2 = 0.98$) than with the radical-scavenging activity ($R^2 = 0.56$). It could be estimated that the phenolic compounds present in the guava leaves played an important role in antioxidant activity, directly through the mechanism of reduction of oxidized intermediates in the chain reaction. The leaf of guava has been reported to contain many different kinds of phenolic compounds. Matsuo, Hananure, Shimoi, Nakamura, and Tomita (1994) identified (+)-gallocatechin from the methanol extract and demonstrated that it had an antimutagenic action against UV-induced mutation in *Escherichia coli*. Huang and Zhang (2004) reported that the major components in the ethanol extract of guava leaves included triterpenic acids, carbohydrates and polyphenols. Liang, Quian, and Yao (2005) analyzed several polyphenolic compounds from the leaves of guava by HPLC-UV analysis and HPLC mass spectrometry. It was reported that these were gallic acid, quercetin, procatechuic acid, chlorogenic acid, caffeic acid, kaempferol and ferulic acid. From the two flavonoids

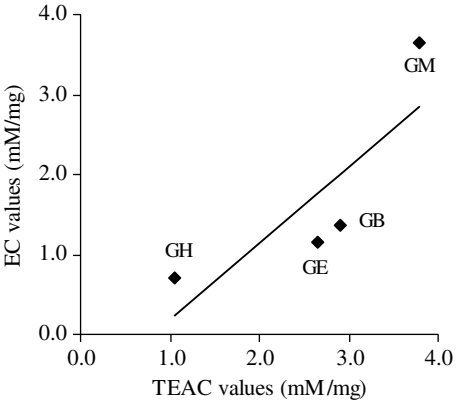

Fig. 3. Correlation of TEAC and EC values of guava leaf extracts from methanol (GM), butanol (GB), ethyl acetate (GE) and hexane (GH).

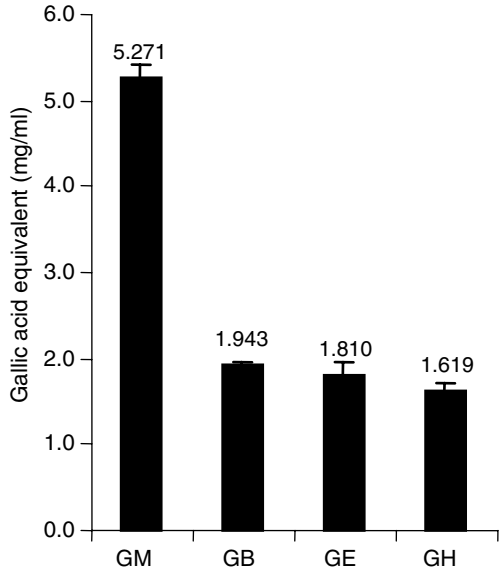

Fig. 4. Total phenolic contents of guava leaf extracts from methanol (GM), butanol (GB), ethyl acetate (GE) and hexane (GH).

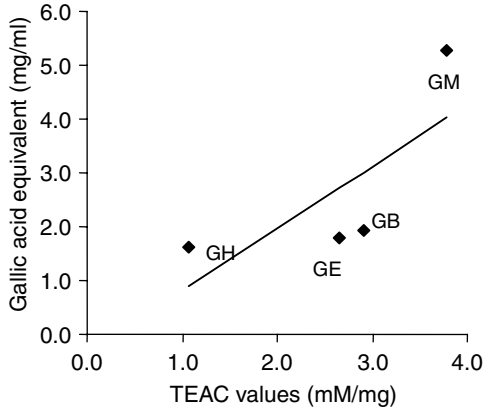

Fig. 5. Correlation of TEAC values and phenolic contents of guava leaf extracts from methanol (GM), butanol (GB), ethyl acetate (GE) and hexane (GH).

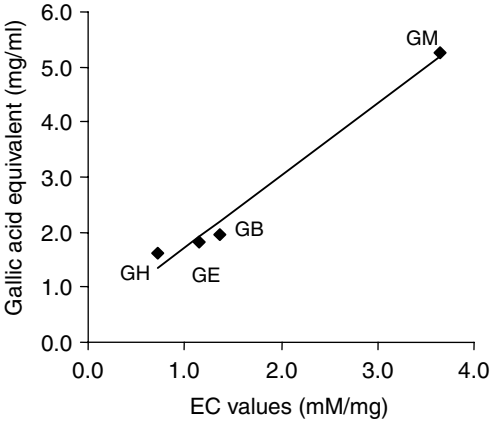

Fig. 6. Correlation of EC values and phenolic contents of guava leaf extracts from methanol (GM), butanol (GB), ethyl acetate (GE) and hexane (GH).

(quercetin and kaempferol), four flavonoid glycosides (quercetin 3-*O*-α-L-arabinoside, quercetin 3-*O*-β-D-glucoside, quercetin 3-*O*-β-D-galactoside and kaempferol-glycoside) were identified. According to their polyphenolic functional groups whose polarity was similar to that of methanol, these active compounds could be completely dissolved in the solvent. These compounds were supposed to play an important role in the antioxidant activity of guava leaves. The lower correlation between TEAC values and the phenolic contents in guava leaves indicated that not only the phenolic compounds were involved in the antioxidant activity through this pathway. There might be some effects involving other active compounds. Although many known compounds were identified, it is not known how much they contributed to the antioxidant activity present in the guava leaves. This present study suggests possible synergistic or competitive antioxidant action among such active compounds. Therefore, further study should be carried out to identify the predominant compounds of the guava leaves with respect to their mechanisms and synergistic actions.

4. Conclusions

Our data have demonstrated the wide range of antioxidant activity among different species and parts of the plants commonly found in Thailand. Among these, guava leaves showed the highest antioxidant activity. Further evaluation of guava leaves, using different solvents has demonstrated that the highest antioxidant activity was in the methanol fraction. The high antioxidant activity of such extracts was attributed to radical-scavenging and reducing mechanisms. The results demonstrated that the phenolic contents existing in guava leaf fractions play an important role in the antioxidant activity directly through the reducing activity mechanism, with some mutual action among the active compounds through free radical-scavenging activity.

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